[2] D.L. Court, J.A. Sawitzke, L.C. Thomason, Genetic engineering using homologous recombination, Annu. Rev. Genet. 36 (2002) 361–388.

doi:10.1016/j.bbabio.2012.06.292

14P14

Pet117 — Assembly factor of cytochrome *c* oxidase

B. Kulawiak, N. Gebert, C. Schütze, A. Schulze-Specking, N. Wiedemann, N. Pfanner
Institute for Biochemistry and Molecular Biology, University of Freiburg, Stefan Meier Str. 17, 79104 Freiburg, Germany
E-mail: bogusz.kulawiak@biochemie.uni-freiburg.de

Cytochrome *c* oxidase is assembled of 13 subunits in mammals and 11 subunits in yeast *Saccharomyces cerevisiae*. The core subunits of complex IV encoded by mitochondrial genome are conserved among the *Eukaryotes* and contain active centers crucial for enzyme activity.

The assembly process of cytochrome *c* oxidase requires large number of assembly factors and includes formation of several intermediates. The crucial step in the assembly of the complex IV is formation of active centers in core subunits including copper and heme insertion into Cox1.

Here we show that Pet117, a conserved protein with a single transmembrane domain is a crucial assembly factor of cytochrome *c* oxidase.

We observed that deletion of yeast Pet117 leads to loss of complex IV assembly and formation of *'petit'* colonies by cells grown on YPG medium. We also noticed decreased levels of several structural subunits of complex IV including Cox1 and Cox2 in Pet117 deficient strain. The wild type phenotype could be restored after complementation of the deletion strain with the plasmid containing Pet117 gene.

Import of radiolabeled Pet117 and carbonate extraction assay revealed that protein localizes in the inner mitochondrial membrane. Moreover, Blue Native gel analysis showed that Pet117 forms protein complexes of 120 kDa, 400 kDa, and above 600 kDa.

Using SILAC approach followed by pull-down experiments with tagged Pet117 we observed strong interaction with copper binding protein Cox11, known assembly factor responsible for Cu delivery to Cox1 subunit. Additionally, detailed analysis of elution fractions with the use of both SDS and Blue Native gels revealed interaction of Pet117 with structural components and assembly factors of complex IV including Cox1, and components of complex III. Similar results were obtained with the use of a strain containing tagged Cox11 protein. Moreover, Pet117-Cox11 interaction was observed in the absence of structural subunits of complex IV and some of assembly factors.

Our results suggest that Pet117 may participate in Cu transfer to Cox11 or cooperate with Cox11 during copper delivery process to Cox1 subunit. However, further analysis is necessary to clarify the exact role of Pet117 in assembly of complex IV.

doi:10.1016/j.bbabio.2012.06.293

14P15

Shewanella oneidensis terminal oxidases

S. Le Laz, A. Kpebe, M. Rousset, M. Brugna-Chiata
Laboratoire de Bioénergétique et Ingénierie des Protéines, CNRS, Aix-Marseille Université, UMR 7281, 31 chemin J. Aiguier, 13402 Marseille cedex 20, France
E-mail: mbrugna@imm.cnrs.fr

In aerobic respiration of prokaryotic and eukaryotic organisms, the reduction of molecular oxygen to water is catalyzed by terminal oxidases, cytochrome *c* or quinol oxidases, which are integral membrane multi-subunit enzymatic complexes pumping protons across membrane. Two types of terminal oxidases are known, the heme-copper oxidases and the cytochrome *bd*-type oxidases. The heme-copper oxidases are classified into three families: type A (mitochondrial like oxidases), type B (ba_3-type oxidases) and type C (cbb_3-type oxidases). The subunit composition of types A and B enzymes differs from one oxidase to another but these heme-copper oxidases always contain the catalytic subunit (subunit I) and a smaller subunit named subunit II [1].

Shewanella oneidensis, a gram-negative proteobacterium, inhabits a wide variety of niches in nature and has the characteristic ability to reduce, in addition to oxygen, a broad spectrum of electron acceptors such as metals, nitrate, thiosulfate, dimethyl sulfoxide, trimethylamine N-oxide, fumarate and azo dyes.

The *S. oneidensis* MR-1 genome sequence analysis revealed the presence of genes coding for enzymes potentially involved in oxygen reduction: two cytochrome *c* oxidases and a cytochrome *bd* quinol oxidase [2]. Based on sequence comparison, we proposed that the oxidase encoded by the genes SO4606-SO4609 is a cytochrome *c* oxidase (Cox) belonging to type A whereas cytochrome *c* oxidase encoded by SO2361–SO2364 is a C-type enzyme (cbb_3-type). The deduced amino acid sequence of Cox revealed that the subunit II contains two *c* type-heme binding motifs, an uncommon feature among type-A oxidases.

In the membrane of *S. oneidensis* MR1, we detected a cytochrome *c* oxidase activity. The preliminary study of the wild type strain as well as of three single oxidase deletants (lacking the gene encoding the catalytic subunit) shows that the cbb_3-type oxidase as well as the cytochrome *bd* quinol oxidase is present in the membrane of *S. oneidensis* MR-1, in our culture conditions (vigorously shaked 100 mL flask).

References

[1] J.A. Garcia-Horsman, B. Barquera, J. Rumbley, J. Ma, R.B. Gennis, The superfamily of heme-copper respiratory oxidases, J. Bacteriol. 176 (1994) 5587–5600.
[2] J.F. Heidelberg, I.T. Paulsen, K.E. Nelson, et al., Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*, Nat. Biotechnol. 20 (2002) 1118–1123.

doi:10.1016/j.bbabio.2012.06.294

14P16

Yeast mitochondrial cytochrome *c* oxidase: Effect of mutations in the hydrophilic channels within Cox1 and the adjacent supernumerary subunit Cox5A/B

A. Maréchal, B. Meunier, R. Dodia, P.R. Rich
Glynn Laboratory of Bioenergetics, Institute of Structural and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK
Centre de Génétique Moléculaire du CNRS, UPR 3404, avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France
E-mail: a.marechal@ucl.ac.uk

Yeast *Saccharomyces cerevisiae* has recently been developed for facile purification of mutant forms of a mitochondrial cytochrome *c* oxidase (CcO) [1]. It is composed of eleven subunits, three forming its catalytic core (Cox1, 2 and 3) and eight others, of unknown role, which are homologous to supernumerary subunits of mammalian CcOs [2]. One of these has two isoforms, Cox5A and 5B, which have 68% sequence identity and are selectively expressed under normoxic

or hypoxic conditions [3]. Mutations have been introduced into yeast mitochondrial DNA to target residues in hydrophilic regions of Cox1. In addition, a series of mutants was constructed in which specific Cox5 isoforms were expressed or in which residues of Cox5A were mutated to match those in 5B. The ability of all mutant strains to assemble a stable C*c*O and grow on respiratory medium was assessed and their O_2-consumption rates were measured. Selected C*c*Os with mutations in the D and H channels were purified to investigate their effects on C*c*O functioning. Mutation of D channel residues E243D and I67N allowed the definitive assignment of E243 as the essential protonated carboxyl group that appears in CO photolysis [4] and redox FTIR difference spectra of mitochondrial forms of C*c*O. Effects of mutations in the H channel and in Cox5 are discussed in terms of the role of the H channel and the potential of supernumerary subunit isoforms to influence catalysis.

References

[1] B. Meunier, A. Maréchal, P.R. Rich, Construction of histidine-tagged yeast mitochondrial cytochrome *c* oxidase for facile purification of mutant forms, Biochem. J. 444 (2012) 199–204.
[2] A. Maréchal, B. Meunier, D. Lee, C. Orengo, P.R. Rich, Yeast cytochrome *c* oxidase: a model system to study mitochondrial forms of the haem-copper oxidase superfamily, Biochim. Biophys. Acta Bioenerg. 1817 (2012) 620–628.
[3] P.V. Burke, D.C. Raitt, L.A. Allen, E.A. Kellog, R.O. Poyton, Effects of oxygen concentration on the expression of cytochrome *c* and cytochrome *c* oxidase genes in yeast, J. Biol. Chem. 272 (1997) 14705–14712.
[4] A. Maréchal, B. Meunier, P.R. Rich, Assignment of the CO-sensitive carboxyl group in mitochondrial forms of cytochrome *c* oxidase using yeast mutants, Biochim. Biophys. Acta Bioenerg. (2012)doi:10.1016/j.bbabio.2012.03.036.

doi:10.1016/j.bbabio.2012.06.295

14P17

The function of Cox7a1 for brown fat thermogenesis (S14 terminal oxidases)

Stefanie Maurer, Tobias Fromme, Martin Klingenspor
Molecular Nutritional Medicine, ZIEL Research Center for Nutrition and Food Sciences, Technische Universität München, Freising, Germany
E-mail: stefanie.maurer@tum.de

Cox7a1 is one of two isoforms of subunit 7a in cytochrome *c* oxidase (COX), which is the terminal respiratory chain enzyme in mammals. This protein is predominantly expressed in tissues with high aerobic capacity and a large number of mitochondria such as heart and skeletal muscle. Cox7a1 protein expression is also present in brown adipose tissue (BAT) — a specialized tissue, providing adaptive non-shivering heat production in order to maintain normothermia in mammals exposed to low environmental temperatures. Importantly, thermogenesis in BAT is mediated by an increased COX activity and elevated levels of uncoupling protein 1 (UCP1), resulting in a shift from coupled towards uncoupled proton translocation in mitochondria. In a previous study we have shown that Cox7a1 protein levels in BAT are strongly increased in cold-exposed (4 °C) mice compared to room-temperature (20–22 °C) acclimated animals [1]. For this reason, we hypothesize that Cox7a1 replaces Cox7a2 in BAT to serve as more active isoform of COX-subunit 7a, thereby increasing COX activity and BAT thermogenic capacity at low ambient temperatures. To study this phenomenon, we employ Cox7a1 knockout mice.

In our study, we compare BAT function in wildtypes and knockouts that were either housed at 5 °C (cold-exposed) for variable periods (4–15 days) or constantly kept at room-temperature (control group). Cox7a1 and Cox7a2 mRNA were detected in wildtypes of all groups. Cold-exposed wildtypes exhibit an increased Cox7a1 to Cox7a2 ratio (>1), indicating BAT to be one of the major sites of Cox7a1 expression. Although cold-exposure leads to elevated COX activity in BAT as expected, differences in the oxygen consumption of complex IV between wildtypes and Cox7a1 knockouts were not detected within the groups. Since measurement of COX activity was so far performed in tissue homogenates, thus measuring the oxygen consumption of solubilized complex IV, upcoming experiments will predominantly focus on BAT function and activity *in vivo* in wildtypes and knockouts.

Reference

[1] F. Forner, C. Kumar, C.A. Luber, T. Fromme, M. Klingenspor, M. Mann, Cell Metab. 10 (2009) 324–335.

doi:10.1016/j.bbabio.2012.06.296

14P18

Electrochemical analysis of cytochrome ba_3 from *T. thermophilus* immobilized on gold nanoparticles

Frederic Melin[1], Thomas Meyer[1], Julien Gross[1], Sylvia K. Choi[2], Robert B. Gennis[2], James A. Fee[3], Petra Hellwig[1]
[1]*Institut de Chimie UMR 7177, Laboratoire de spectroscopie vibrationnelle et electrochimie des biomolecules, Université de Strasbourg, 1 rue Blaise Pascal, 67070 Strasbourg, France*
[2]*Center for Biophysics and Computational Biology, University of Illinois, Urbana-Champaign, IL 61801, USA*
[3]*Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, USA*
E-mail: fmelin@unistra.fr, hellwig@unistra.fr

Protein film voltammetry has been established as a very convenient method for studying electrochemical and catalytic properties of redox enzymes [1]. It is a real challenge, however, to immobilize on the surface of electrodes a large amount of integral membrane proteins and to establish a good electronic communication with the enzyme cofactors while maintaining a suitable environment for these large complex structures.

We are using 3D gold nanoparticle networks as the conductive support of immobilization for membrane proteins and fragments [2]. The gold nanoparticles, indeed, have been shown to mediate the long-range electron transfer between the electrode and the cofactors and their high surface to volume ratio allows at the same time a high coverage of the electrode with proteins [3].

We will report the electrochemical analysis under aerobic and anaerobic conditions of cytochrome ba_3 from *Thermus thermophilus* [4] immobilized on gold nanoparticles. The influence of the pH will be discussed. The structure of the immobilized membrane proteins will be carefully monitored by Surface-Enhanced vibrational spectroscopies, both IR (SEIRAS) and Raman (SERRS). The studies will be completed by FTIR difference spectroscopy to show the influence of the heme propionates in the pH dependence.

References

[1] F.A. Armstrong, J. Hirst, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 14049–14054.
[2] T. Meyer, J. Gross, C. Blanck, M. Schmutz, B. Ludwig, P. Hellwig, F. Melin, J. Phys. Chem. B 115 (2011) 7165–7170.